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Antibacterial Activity of Various ZnO Nanostructures on Pathogenic Bacteria Found in Selaiyur Lake, Tamil Nadu

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Abstract

Water is the elixir of life and globally the imperative need of the hour is uncontaminated water. Water has a great impact on every aspect of human life and with increasing environmental pollution and rapid growth of population, the need for developing technologies to remove pathogenic bacteria from the water bodies has become inevitable. This research aims to study the effect of synthesized ZnO nanostructures on a few very common gram-positive and gram-negative bacteria like Klebsiella, Staphylococcus, Proteus and Bacillus found in Selaiyur Lake, Tambaram, Tamil Nadu. Zinc oxide nanoparticles used for the anti-bacterial study were synthesized by dry mechano- chemical and sol-gel methods. The SEM images of the samples show formation of spherical, flower shaped, nut shaped and hexagonal disc shaped nanostructures. The XRD analyses show the formation of pure hexagonal wurtzite crystalline structures. The particle sizes of the synthesized samples were found to be in range of 15- 90 nm. The Lake water samples taken for studies underwent serial dilution using saline solution under sterile conditions. By the method of spread plate the bacterial colonies are observed. The experimental results show that the synthesized zinc oxide nanoparticles have appreciable antibacterial properties. Using the gram stain test, both gram-positive and gram-negative bacteria were found in the sample. The nanoparticles were found to be resistant against both types of bacteria. The spherical nanostructures with ZnO nanoparticles of 16-19 nm sizes synthesized by dry- mechano chemical method was found to be more effective against all the four types of bacteria studied. The size and surface morphology effect of ZnO nanostructures on bacterial growth was also studied in comparison with commercial Bulk ZnO nanopowder.

Keywords: *Dry Mechano-chemical; Pathogenic bacteria; Sol-gel; ZnO nanostructures.*

1. INTRODUCTION

In this present era of technology, Nano science has taken the centre stage in modern material science. It is a branch of science that is capable of providing innovative applications in almost every walk of life, through the exploration of the uniqueness of materials at the nanoscale level (1-100nm) (Viswanataha, 2014; Jones *et al.* 2008). The most important aspect of nanomaterial is that these nano-sized materials bring about a new quantum mechanical effect because of its vast increase in surface area to volume ratio, which results in higher catalytic activity (Tolochko,). Changes in the size, composition and morphologies of metal oxide nanoparticles like Zinc Oxide (ZnO), Silver, Titanium Oxide (TiO), MgO, CuO,etc., can modify their chemical, mechanical, structural, electrical and optical properties. Of the mentioned metal oxides, ZnO taken in our study is marked as one of the vastly studied and used because of its bio-compatible, eco-safe, simpler and cost effective methods (Yanping Xie *et al.* 2011).

Zinc oxide (ZnO) has become the epitome of recent metal oxide research with its variety of morphologies and sizes giving way to many applications. It is an inorganic compound used in many materials or end products like plastic, ceramics, glass, etc. in powdery form, it has a strong ionic bond and crystallizes into three crystal forms namely: the hexagonal wurtzite, the cubic zinc blende and the cubic rock salt (Gertrude Neumark and Kuskovsky, 2007; Ozgur *et al.* 2005). Zinc Oxide has a wide band gap of 3.3eV with a high exciting binding energy (60meV)

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(Fab abd Ky, 2005). Zinc Oxide among all other metal oxides or materials has the richest nanostructures and properties exhibiting a large range of different morphologies like nano-sheets, nano-tubes, nano-rods and nano-combs which are implemented on a variety of applications (George *et al.* 2009; Yahya *et al.* 2010) like anticancer studies (Selvakumari *et al.* 2015), cosmetics etc. The properties and versatility of ZnO pave a way to synthesis ZnO nano particles using various methods like Sol-Gel method, dry-mechano chemical method, hydrothermal, metal catalysed, vapour emulsion precipitation liquid-solid growth, precipitation method and so on (Janotti and Van de Walle, 2009). By controlling the synthesis parameters such as the temperature, reactant ratios, pH and other environmental parameters a better yield of the desired nano particles can be achieved. Optimization of geometry, structure, morphology and the electronic, mechanical and optical properties of the nanometre-size systems are of fundamental importance for the design of nanostructures (Wang, 2004; Song *et al.* 2011; Padmavathy and Vijayaraghavan, 2008).

In the present study, the anti-bacterial properties of ZnO nanoparticles against some of the pathogenic bacteria that are present in Lake Water. Even though the earth is covered with 70% of water, its high content of salt makes it unfit for human consumption and most of the water bodies like sea, rivers and lakes are highly polluted by dumping of wastes from industries and household which increases the number of harmful pathogenic bacteria like *Bacillus, Proteus* and so on. Bacterial diseases are serious health problems that have drawn the public attention worldwide and are a human health threat that extends to economic and social complications. With regard to this, a case study has been done on the various bacteria found in Selaiyur Lake, Tambaram, Tamil Nadu, India and the experimental work on the antibacterial property of ZnO was done.

Serial dilution method and spread plate method are employed for growing the lake water bacteria which is then followed by the well diffusion method incorporating the ZnO nanoparticles into the well smeared by the bacteria of which after incubation the inhibition zones are observed.

2. MATERIALS & METHODS

2.1 Synthesis of ZnO by Dry- Mechano Chemical Method

Dry mechano chemical method is one of the simple, less sophisticated methods adopted for the synthesis of ZnO 1 and ZnO 1.5.It involves manual grinding of reactants followed by calcinations. Zinc Acetate di hydrate and Oxalic acid were taken in measured molar concentration and using agate mortar and pestle the mixture was ground for an hour under room temperature conditions. Then the mixture was annealed at 450°C for about another hour.

$$Zn(CH_3COO)_2 \pm 2H_2O + H_2C_2O_4 \pm 2H_2O \rightarrow ZnC_2O_3.XH_2O + CH_3COOH + 2H_2O \quad (1)$$

$$ZnC_2O_4 \pm XH_2O \rightarrow ZnO + CO + CO_2 + XH_2O \quad (2)$$

Nomenclature

ZnO 1	ZnO nanoparticles synthesised by dry mechano- chemical method with1:1 molar ratio of the reactants Zinc acetate and oxalic acid
ZnO 1.5	ZnO nanoparticles synthesised by dry mechano- chemical method 1:1.5 molar ratio of the reactants Zinc acetate and oxalic acid
PEG	Polyethylene glycol
ZnO 2000	ZnO nanoparticles synthesised by sol-gel method by using PEG 2000 as a surfactant.
ZnO 4000	ZnO nanoparticles synthesised by sol-gel method by using PEG 4000 as a surfactant.
DMSO	Dimethyl Sulpha Oxide.
Sample A	100mg of ZnO1 in 1 ml of DMSO.
Sample B	100mg of ZnO1.5 in 1ml of DMSO.
Sample C	100mg of ZnO2000 in 1ml of DMSO.
Sample D	100mg of ZnO4000 in 1ml of DMSO.
Sample E	50mg of ZnO1 in 1ml of DMSO.
Sample F	50mg of ZnO1.5 in 1 ml of DMSO
Sample G	50mg of ZnO2000 in 1ml of DMSO
Sample H	50mg of ZnO4000 in 1ml of DMSO

General reagent grade (Merck make) Zinc acetate dihydrate $Zn(CH_3COO)_2 \pm 2H_2O$ and Oxalic acid, $H_2C_2O_4 \pm 2H_2O$ were taken in equal molar ratio of 1:1, i.e., 0 ± 01 M of zinc acetate with 0±01M of oxalic acid in an agate mortar and continuous grinding was carried out manually for one hour. As the reaction proceeded, the smell of acetic acid was sensed in the first 10 minutes, with a formation of paste like intermediate product. On further grinding in room temperature for another 50 minutes dry free flowing, zinc oxalate powder was obtained. The resulting white powder was annealed in muffle furnace at 450 °C for about an hour. Similarly, ZnO1.5 is prepares with zinc acetate and oxalic acid in the molar concentration ratio of 1:1.5. After the heat treatment the obtained nano ZnO powder was cooled to room temperature and taken for XRD, and SEM analysis.

2.2 Synthesis of ZnO by Sol-Gel method

Zinc acetate was used as a precursor, ethanol as a solvent and PEG as the surfactant. Ammonium hydroxide was added in order to vary the pH of the solution.13.17gm of Zinc acetate is added to 60ml of Ethanol and stirred using a magnetic stirrer for two hours at room temperature until a homogenous clear solution was obtained. Ammonium hydroxide was added to this solution until the pH of the solution turned 7.18 and the solution was further stirred two hours at room temperature. A milky white solution was obtained. The obtained solution was divided into two equal parts. PEG 2000 was mixed with 100 ml distilled water to obtain a gel, and this was mixed to one part of the obtained solution. Similarly, the same was done using PEG 4000. These two prepared sol-gels were allowed to age for four days. Then the aged sol gel was annealed at 500 °C in a muffle furnace for two hours to obtain the nanostructured Zinc oxide powder.

2.3 Antibacterial Study

The water samples were collected from the Selaiyur Lake, Tambaram, Tamil Nadu. Serial dilution was the first step wherein using a sterile conical flask 1ml of the water sample was diluted in 9ml of saline solution in a test tube to make a total solution of 10ml. Further, 1ml of this sample was diluted by another 9ml of saline solution; the procedure was carried for dilution for up to 10^{-6} concentration of the sample. Later by spread plate method 0.1 ml of the sample is swabbed using a sterile glass rod in a nutrient agar medium and incubated in 37 °C for 24 hours. The same procedure was followed for the various concentrations of the solution. After 24 hours, the formations of distinguished colonies of bacteria were found. These colonies of bacteria were isolated and by various bio chemical tests the colonies were identified. The first test involves checking if the bacteria are gram positive or gram negative. After the biochemical tests the bacteria were identified as Staphylococcus, Proteus, Bacillus, and Klebsiella. The final step is the well diffusion method, wherein the nutrient agar was set into sterilized petri dish and using a well cutter, wells were cut into the nutrient agar medium. Nano ZnO powder dissolved in di methyl sulpha oxide of different concentrations was diffused into these wells and these were incubated at 37 °C overnight. The Zone of inhibition observed in the wells show the antibacterial effect of the nano ZnO against the bacteria.

3. RESULTS & DISCUSSION

The crystal structure and surface morphology of the synthesized ZnO nanoparticles were characterized using the XRD, and SEM analysis. The figs1 (a) and 1(b) show the XRD pattern of the standard ZnO (JCPDS35- 1451) along with diffraction pattern of the samples ZnO1, ZnO 1.5, ZnO 2000 and ZnO 4000. All peak positions and relative peak intensities of the ZnO product agree well with those of the standard XRD pattern and no characteristic peaks of impurities, such as ZnC_2O_4, were observed, indicating that the ZnO nanoparticles product is of high purity. The phase structure of ZnO nanoparticles belongs to a wurtzite structure. The average crystal sizes of the particles were calculated by Scherrer's equation given by equation (3).

$$D = k\left(\frac{\lambda}{\beta cos\theta}\right) \quad (3)$$

Where, D – mean size of crystalline domains in nm; k-shape factor = 0.9, λ is the X-ray wavelength; β is the line broadening at half the maximum intensity (FWHM) and θ is the Bragg angle.

Table 1. Average Crystal size of ZnO 1

Peak	FWHM	2Θ	Size (D in nm)	h	k	l
1	0.37379	31.72366	22	1	0	0
2	0.41197	34.39124	20	0	0	2
3	0.38432	36.4432	22	1	0	1
4	10.62528	47.5376	18	1	0	2

Table 2. Average Crystal size of ZnO 1.5

Peak	FWHM	2Θ	Size (D in nm)	h	k	l
1	0.53569	31.87414	15	1	0	0
2	0.52681	34.5554	16	0	0	2
3	0.57177	36.36114	14	1	0	1
4	0.68784	47.61969	12	1	0	2

Table 3. Average Crystal size of ZnO 2000

Peak	FWHM	2Θ	Size (D in nm)	h	k	l
1	0.244	31.665	34	1	0	0
2	0.264	34.432	31	0	0	2
3	0.309	36.292	28	1	0	1
4	0.330	47.674	27	1	0	2

Table 4. Average Crystal size of ZnO4000

Peak	FWHM	2Θ	Size (D in nm)	h	k	l
1	0.244	31.965	35	1	0	0
2	0.264	34.445	32	0	0	2
3	0.251	36.306	34	1	0	1
4	0.309	47.450	29	1	0	2

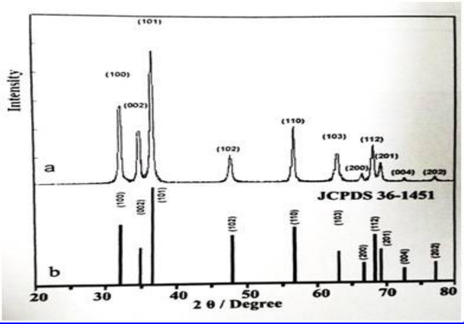
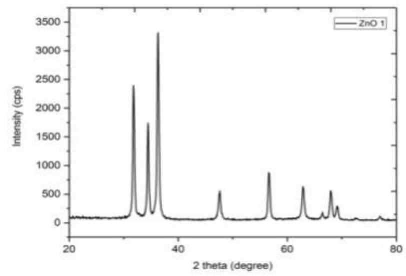
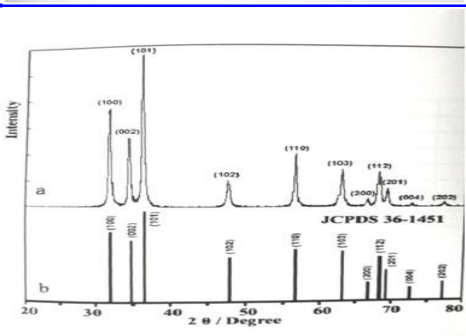
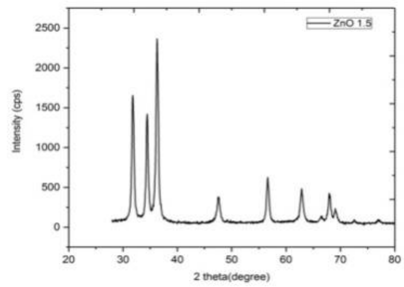
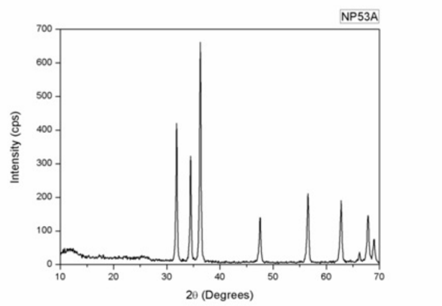
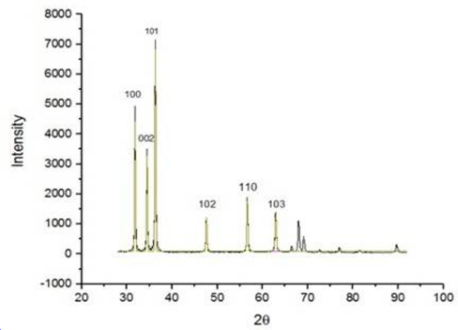
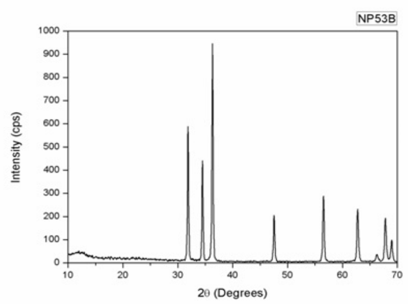
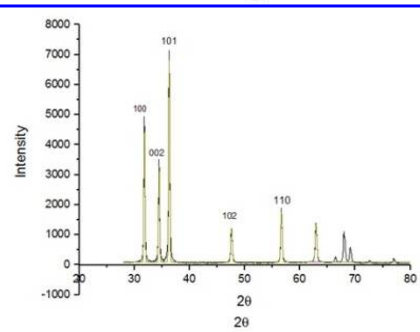
Sample	XRD (Trial 1)	XRD (Trial 2)
ZnO 1		
ZnO 1.5		
ZnO 2000		
ZnO 4000		

Fig. 1: (a) and (b) - The XRD pattern of Samples in Trial 1 and Trial 2

The SEM images of all the four ZnO nanoparticles (both trials) are as shown in fig. 2(a) and 2(b). The pictures indicate that the ZnO 1 nanoparticles have small spherical shaped morphology whereas ZnO 1.5 nanoparticles have very geometrically proportional nano-rods grown in a flower pattern. The variations in the molar ratio of the reactants zinc acetate di hydrate and oxalic acid greatly seem to affect the morphology of the nanoparticle. It is also observed that the distribution of sizes or morphology of the nanoparticles is very uniform. The repeatability of size and morphology of the nanoparticles were also tested in this method of synthesize.

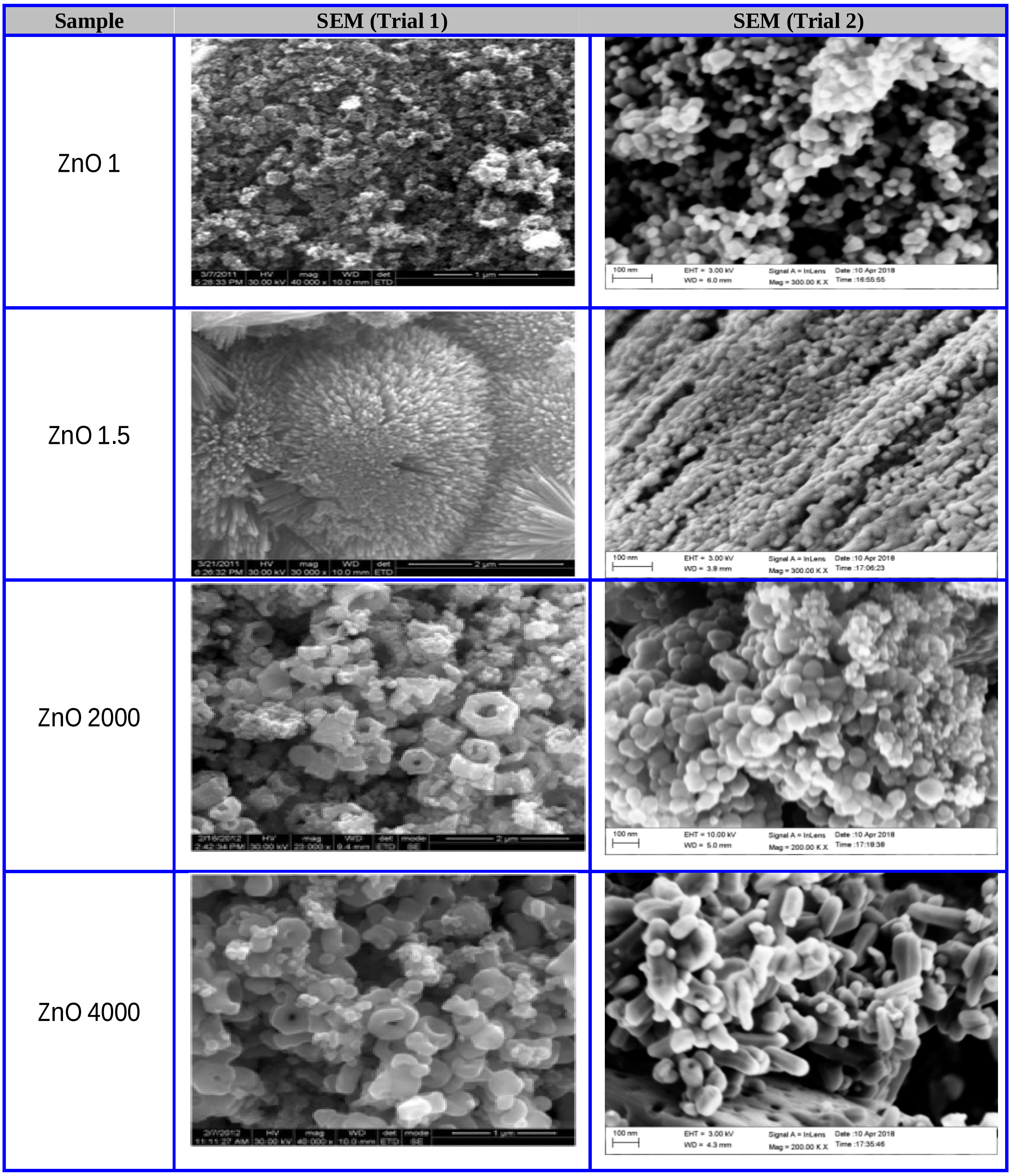


Fig. 2: (a) SEM Images of ZnO samples trial 1; (b) trial 2

The SEM image of ZnO 2000 has a bolt and small cylindrical shaped morphology of trial 1 and 2 respectively. The SEM images of ZnO synthesized in the presence of PEG of varying molecular weights at different magnifications are shown. The variation in the molecular weight of PEG did not seem to affect the morphology to a greater extent. The shape of the ZnO with PEG 2000 is well defined when compared to the ZnO with PEG 4000. The added water molecules in the case of PEG 4000 may be the reason for the bolt shape of ZnO. Water in glycols plays an important role in the formation of the characteristic structures, and by controlling the volume ratio of PEG to water one can vary the morphology of ZnO nanoparticles. Higher water content in PEG results in hollow spheres (bolt), and the cavity size increases with decreasing PEG to water ratio. The distribution of ZnO nanoparticles seems to improve and the particles aggregate less as the molecular weight of the surfactant decreases.

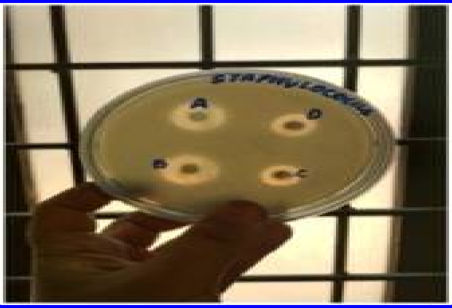
Bacteria	Zone of Clearance (Trial 1)	Zone of Clearance(Trial 2)
Staphylococcus		
Bacillus		
Proteaus		
Klebsiella		

Fig. 3: (a) Antibacterial activity studies- trial 1; b) Antibacterial activity studies- trial 2.

Table 5. Diameter of zone of clearance observed from fig. 2(a) and 2 (b)

Bacteria	Diameter of Zone of inhibitionin cm								Bulk ZnO
	ZnO 1		ZnO 1.5		ZnO 2000		ZnO 4000		
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trail 2	Trail 1	Trail 2	
Staphylococcus	1.6	2.0	1.6	1.9	1.4	1.5	1.3	1.3	1.1
Bacillus	2.0	2.3	2.0	1.9	2	1.7	1.6	1.3	1.1
Klebsiella	1.5	1.5	1.3	1.3	1.5	1.4	1.2	1.2	1.1
Proteus	2.3	2.0	1.7	1.3	2.3	1.6	1.7	1.7	1.1

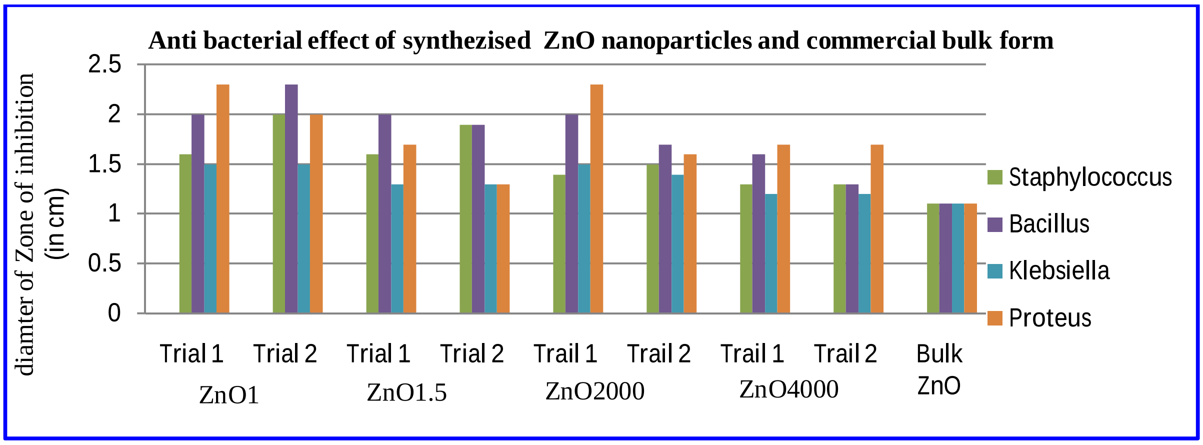


Fig. 4: Bar graph showing the antibacterial activity of the various ZnO nanoparticles of trial 1 and trial 2 and Bulk ZnO

From the measurement of the diameter of the zone of clearance seen in fig. 3(a) and 3(b) it can be observed that all the four samples of ZnO nanostructures exhibit noticeable antibacterial effect than the commercially obtained Bulk ZnO sample. It is also evident that the ZnO1 sample with spherical morphology has a greater antibacterial effect on almost all the four kind of Bacteria studied in both the trails. The antibacterial effect of ZnO 1 sample, consisting of spherical shaped nanostructure with the average grain size 12-16nm is significantly higher in both the trials compared to the other nanostructures.

Table 6. Diameter of zone of clearance (cm) based on two concentration(mg/ml).

Bacteria	Concentration in mg/ml							
	ZnO 1		ZnO 1.5		ZnO 2000		ZnO 4000	
	100 mg/ml	50 mg/ml	100 mg/ml	50 mg/ml	100 mg/ml	50 mg/ml	100 mg/ml	50 mg/ml
Staphylococcus	1.9	1.4	1.7	1.3	1.3	1.1	1.2	1.1
Bacillus	2.0	1.6	1.8	1.5	1.3	1.0	1.3	1.2
Klebsiella	1.5	1.2	1.3	1.1	1.4	1.2	1.6	1.2
Proteus	1.5	1.3	1.3	1.1	1.2	1.0	1.4	1.3

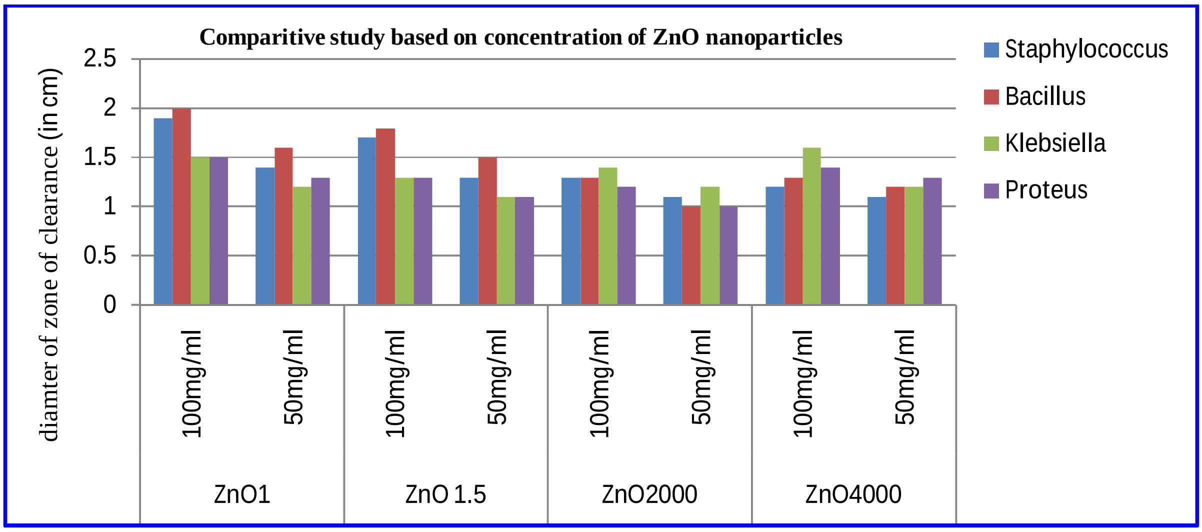


Fig. 5: Antibacterial activity of ZnO trial 1 and ZnO trial 2 based on concentration

It is observed from fig. 5, that the antibacterial activity also depends upon the concentration of the nanoparticles. Hence, it is seen that more the concentration of ZnO nanoparticles defused into the better is the antibacterial effect.

4. CONCLUSION

ZnO nanoparticles is "generally considered safe" for human consumption. It possesses unique properties and excellent stability with long life compared to other organic based disinfectants that stimulate its use as an antibacterial agent. In comparison to other metal oxide nanoparticles such as Fe_2O_3, MgO, AuO, etc., ZnO nanoparticles are less toxic, have a higher antibacterial activity and can be prepared in a cost effective way. Nanoparticles can be synthesized through various methods but from this study, it is observed that ZnO nanoparticles can be obtained through the two simple methods dry-mechano chemical and sol-gel method. From the dry mechano chemical method, the nanoparticles are found to be highly pure, poly crystalline nature with considerable small sizes of spherical and flower shaped morphology. This method is cost effective and yields particles with sizes ranging from 12-27 nm. Similarly sol-gel method yields pure, hexagonal shaped nanoparticles that are distributed uniformly with sizes ranging from 25-35nm.Sizes and morphology of the ZnO nanoparticles can be altered or varied with the change in the molar ratios of the reactants zinc acetate and oxalic acid or the surfactants. From the synthesis of ZnO nanoparticles in trail 1and trail 2, it is seen that both yielded particles with almost similar shape and size, this shows the repeatability of the synthesis process. From the XRD analysis, the average grain size of the particles were calculated by Sherrer's formula.It can be seen that dry-mechano chemical method is an efficient method and produce good, pure ZnO nano sized particles.

Furthermore, the study of the anti-bacterial properties of ZnO nanoparticles shows the effectiveness of the particles on all the four identified bacteria *Bacillus, Staphylococcus, Klebsiella* and *Proteus.* The concentration of the nanoparticles diffused in the wells also plays a vital role in contributing to the antibacterial property of the nanoparticles. Hence, greater the concentration better is the effect. The uniformity and size of the ZnO nanoparticles (purity of the particles) also has an effect on the antibacterial activity, as the sizes are comparable to size of the cell wall and cytoplasm of the bacteria making ZnO1 more effective than the other three types of nanoparticles.

Conflict of interest

The authors declare that there are no conflicts of interest. This study mainly focuses on the antibacterial effect of various ZnO nanostructures synthesized in our lab on the pathogenic bacteria identified in the Selaiyur Lake in Tambaram, Tamil Nadu, India. Our research is mainly the synthesis of nanostructures and their applications to environmental problems and we do not receive any financial supports or any other relationship with other people or organizations.

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